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Methods

Study sites

Thirteen lakes (fourteen stations) in Minnesota and Wisconsin (Fig. 1, Table 1) were sampled during the ice cover season in late winter-early spring and again during the summer stratified season. Lakes were chosen to represent a range of physical, chemical and biological conditions in order to assess how winter and summer conditions differ in diverse north temperate lakes. The two Lake Superior stations include a moderate depth site near Madeline Island and a shallower location in Chequamegon Bay (Fig. 1). We categorized study sites according to the lake total phosphorus, dissolved organic carbon, and chlorophyll *a* concentrations into blue (oligotrophic), green (eutrophic) and brown (dystrophic) lakes (Williamson *et al*., 1999; Webster *et al*., 2008; Leech *et al*., 2018). Lakes in our study with integrated values of TP concentrations ≤ 1 μM L^{-1} (except for Portsmouth lakes), DOC ≤10 mg L^{-1}, and chl *a* ≤5 μg L^{-1} were assigned to blue (oligotrophic) lakes. Brown lakes had integrated values for TP >0.5 but ≤1.3 μM L^{-1}, DOC >10 mg L^{-1}, and chl *a* ≤10 μg L^{-1}. Green lakes had TP concentrations ≥1.3 μM L^{-1}, DOC ≤20 mg L^{-1}, and chl *a* >10 μg L^{-1} (Table 1).

Sample collection

Sampling was conducted from the surface of the ice in March and from a small boat during the ice-free period in July. During both seasons, we collected water column temperature, dissolved oxygen, pH, total dissolved solids, fluorescent dissolved organic matter, and conductivity profiles using a YSI EXO2 multiparameter sonde (YSI In., Yellow Spring, OH, USA), water samples from different depths for chemical analyses and zooplankton samples for determination of community composition and stable isotopes ($\delta^{13}C/\delta^{15}N$) analysis (SIA).

Several physical characteristics were measured during winter and summer periods. During the ice cover period, we visually estimated a percentage of snow cover on the ice. Average snow depth was determined from measurements of 5 locations around the sampling site. The thickness and properties of ice (layering) were recorded as well. Light attenuation through snow, ice and water was measured with either a LI-COR probe equipped with a quantum LI-192 cosine sensor

(LI-COR Biosciences., NE, Lincoln, USA) or a submersible hyperspectral irradiance sensor (TriOS Ramses, Rastede, Germany). Light attenuation of ice was measured by paired measurements in air and by submersing the light sensor through a hole and placing it as close as possible to the underside of the ice. If any snow was present, measurements were repeated after carefully removing the approximately 1 m^2 of snow. Light attenuation in the water column was determined from measurements of light levels at resolution of 0.5 m from water surface to depths of 5-10 m. We calculated the euphotic depth for each sampling location and period while accounting for light attenuation by the water column as well as by snow and ice cover.

Water samples were collected for chl *a*, total phosphorus (TP), dissolved organic carbon (DOC), and seston $\delta^{13}C$/ $\delta^{15}N$ SIA with a 3.7 L Van Dorn water sampler at several discrete depths in each lake (Table 1). At minimum, water was collected at lake surface (or immediately under the ice in winter) and 0.5 m above lake bottom. Water was collected into 2 L acid-washed bottles and stored in the dark until return to the lab for analyses.

Zooplankton samples were collected using zooplankton net tows (0.5 m mouth diameter, 64 μm mesh size), from 1 m above the lake bottom to the surface to determine the total abundance of crustacean zooplankton. One sample from each sampling date was fixed with 90% ethyl alcohol upon collection and then transferred to 70% ethyl alcohol for storage until taxonomic identification and counting. A second zooplankton sample was cleaned from algae and debris and kept alive for ~2 hours in filtered lake water following collection to allow gut clearance; these zooplankton were then frozen for later determination of bulk C and N content and C/N stable isotope composition.

Lab Analyses

Dissolved Organic Carbon (DOC) samples were filtered through pre-combusted Whatman GF/F filters into pre-combusted 40 ml glass vials. Concentrations of DOC were determined using a Shimadzu TOC-V autoanalyzer (Shimadzu Co., Kyoto, Japan). TP was determined using a potassium persulfate digestion method to convert phosphorus to orthophosphate (Murphy and Riley, 1962; Wetzel and Likens, 1991). Samples were then analyzed using a SEAL Analytical AQ400 autoanalyzer with US EPA119-A method for TP (Murphy and Riley, 1962). Chl *a* was filtered onto 0.2 μm nitrocellulose filters and extracted into 90% acetone solution (Welschmeyer, 1994). After an 18 hours extraction period in the dark, extracts were analyzed using a Turner

Designs 10-AU fluorometer (Turner Design, Sunnyvale, CA) using an excitation wavelength of 436 nm and emission of 680 nm.

Seston samples from different depths were filtered onto pre-combusted Whatman GF/F filters and frozen at -20°C. Afterward, filters were dried overnight and rolled in tin capsules for determination of bulk C and N content and C/N stable isotope composition. Bulk zooplankton samples for C and N stable isotopes analysis were kept at -20°C. Samples were freeze-dried, thoroughly homogenized and weighed into tin capsules. C/N SIA on seston and zooplankton samples was performed using Elemental Analysis – Isotope Ratio Mass Spectrometry (EA-IRMS) at the Large Lakes Observatory facilities. For both seston and bulk zooplankton SIA analysis we used acetanilide, B-2153 soil, B-2153 soil, caffeine, and RM8548 standards and run them repeatedly after every ten samples. The analytical errors calculated on seston replicates for $\delta^{13}C$ and $\delta^{15}N$ were 0.36‰ and 0.53‰, respectively.

Preserved zooplankton samples were subsampled using a Stempel pipette and transferred into a Bogorov chamber for counting. We counted and identified samples using an Olympus SZH10 stereoscopic microscope (at 7x-70x magnification). Identification was based on the zooplankton key from Balcer *et al*. (1984) and Haney *et al*. (2013). Subsamples were identified and counted until at least 300 individuals were processed. We identified adult copepods and cladocerans to species level. Copepodites were distinguished between calanoids and cyclopoids only. Cladocerans were counted without age stage determinations. Nauplii were not separated by stage or taxonomic groups (cyclopoid vs. calanoid) and are included only in the total abundance analysis. They were excluded from community composition and feeding group analyses due to their high density and inability to separate them into those groups. Additionally, we assigned adult species to three feeding groups: herbivores, omnivores and predators based on information from Balcer *et al*. (1984) and Haney *et al*. (2013). We calculated abundance as # L^{-1}.

Data analysis

We used the R (version 3.6.2) software environment for statistical analyses of our data (R Core Team 2017). All graphics were created using the ggplot2 package (Wickham, 2009).

The downwelling attenuation coefficient of PAR (K_D) in water was calculated using the Beer-Lambert Law. K_D of ice ($K_{D\ ICE}$) was calculated following Eq. 1, and where present the K_D of snow ($K_{D\ SNOW}$) was calculated following Eq. 2 where Z_{ICE} and Z_{SNOW} are the depth of ice and snow respectively (m). To account for large seasonal differences in daily incident irradiance,

euphotic depths are not calculated as the depth of 1% surface light but rather the depth where mean daily PAR is 1 μmol m^{-2} s^{-1} which corresponds to the proximate minimum light requirement of phytoplankton (Silsbe *et al*., 2016). Mean daily PAR in the summer and winter (485 and 90 μmol m^{-2} s^{-1}) was determined from remotely-sensed PAR climatology (MODIS Aqua) for our region (90 – 95°W, 40 – 45°N). Euphotic depths in the summer and winter are calculated using Eq. 3 and 4 respectively.

(1) $K_{D\,ICE} = -\log\left(E_{Z\,ICE}/E_0\right) \cdot {Z_{ICE}}^{-1}$

(2) $K_{D\,SNOW} = \left[-\log(E_{Z\,ICE}/E_0) + K_{D\,ICE} \cdot Z_{ICE}\right] \cdot {Z_{SNOW}}^{-1}$

(3) $Summer\ Z_{EU} = -\log\left(1/485\right) \cdot {K_D}^{-1}$

(4) $Winter\ Z_{EU} = -\log\left(1/(90 \cdot exp^{-K_{D\,ICE} \cdot Z_{ICE} - K_{D\,SNOW} \cdot Z_{SNOW}})\right) \cdot {K_D}^{-1}$

We calculated depth-integrated values for selected limnological parameters (chl. *a*, DOC, and TP) using trapezoidal integration (Eq. 5).

(5) DIV = $\sum$(c*i* + cb)/2 × (db - d*i*)/td,

where DIV is the depth-integrated value for a particular variable, c*i* is the sample concentration value at depth *i*, cb the sample concentration value at depth below depth *i*, d*i* the sample depth *i*, db the sample depth below depth *i*, and td the station total depth.

To assess differences between seasons for environmental parameters and zooplankton community characteristics we used paired t-tests with adjusted p-values by using the Holm multiple testing correction. Zooplankton and environmental data were log transformed in order to meet the normality and equal variance assumptions for parametric t-tests. Pearson correlation tests were used to investigate the relationship between environmental parameters during winter and summer across all lakes.

To visualize differences between winter and summer in terms of zooplankton abundance and species composition, we used nonmetric multidimensional scaling (NMDS) from the *vegan* package in R (Oksanen *et al.*, 2018). Community similarity was calculated using the Bray-Curtis dissimilarity metric (*betadisper* function in vegan), generated from species abundance data. Species abundance data was fourth-root transformed in order to reduce the influence of the most abundant taxa (Clarke and Warwick, 2001). We also used permutational multivariate analysis of variance (PERMANOVA; *adonis* function in vegan) to test for significant differences in zooplankton community composition between winter and summer seasons. In order to identify

the species that contributed most to the dissimilarity between seasons, we used similarity percentage analysis (SIMPER; *vegan* package; Oksanen *et al.*, 2018). We set a 40% similarity threshold within groups.

Table 1- Study lake characteristics, including color classification, size, depth, sampling depths, sampling dates.

Lake Color	Lake, State	Lake Size, km^2	Site depth, m	Water sampling depths, m	Summer sampling date	Winter sampling date
Blue	Burntside, MN	28.9	26	0, 5, 14, 25	7/13/15	3/19/15
Blue	La Salle, MN	0.9	60.5	0, 7, 20, 35, 57	7/14/15	3/20/15
Blue	Mille Lacs, MN	536.1	8.5	0, 7	7/10/15	3/18/15
Blue	Pike, MN	2.0	13	0, 6, 12	7/8/15	3/16/15
Blue	Portsmouth, MN	0.5	93	0, 15, 25, 35, 80	7/10/15	3/18/15
Blue	Superior (Chequamegon Bay), WI	82102.6	8	0, 7.5	7/9/15	3/17/15
Blue	Superior (Madeline Ice Road), WI	82102.6	47	0, 15, 30, 45	7/9/15	3/17/15
Brown	Barrs, MN	0.52	6	0, 2, 3.75, 5	7/12/15	3/16/15
Brown	Nels, MN	0.7	8	0, 7	7/13/15	3/19/15
Brown	Side	1.5	10	0, 2, 4, 8	7/23/18	3/1/18
Brown	South Sturgeon	0.8	10	0, 2, 4, 8	7/23/18	3/1/18
Green	Briar, MN	0.3	5.5	0, 4.5	7/12/15	3/16/15
Green	Minnetonka	2.3	10	0, 2, 4, 8	7/16/18	2/21/18
Green	Parkers	0.4	10	0, 2, 4, 8	7/16/18	2/21/18

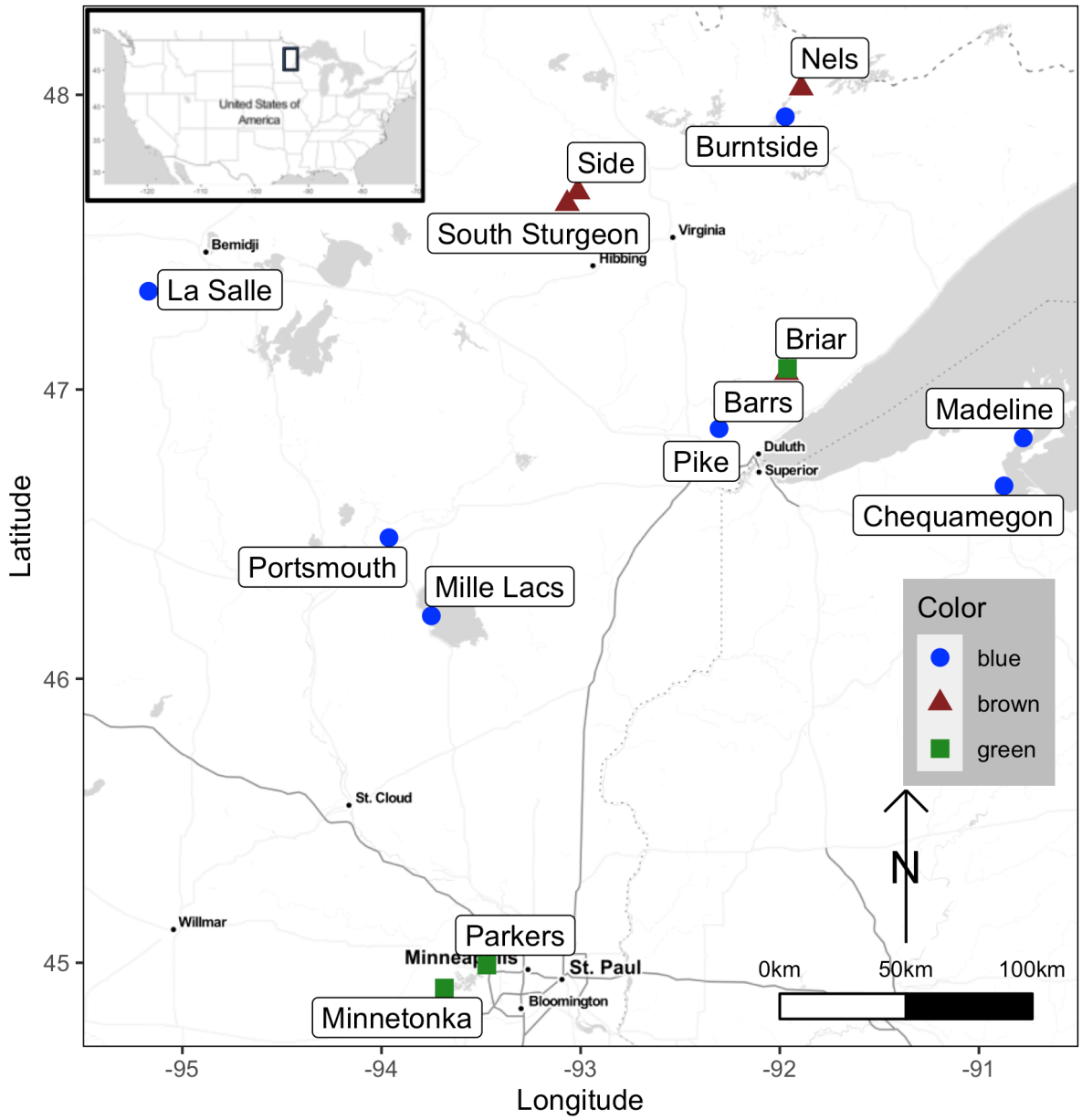

Fig 1 - Map of study locations. Lakes of different water color are indicated by different symbols.

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